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Human neutrophils in auto-immunity

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ABSTRACT

Human neutrophils have great capacity to cause tissue damage in inflammatory diseases via their inappropriate activation to release reactive oxygen species (ROS), proteases and other tissue-damaging molecules. Furthermore, activated neutrophils can release a wide variety of cytokines and chemokines that can regulate almost every element of the immune system. In addition to these important immunoregulatory processes, activated neutrophils can also release, expose or generate neoepitopes that have the potential to break immune tolerance and result in the generation of autoantibodies, that characterise a number of human auto-immune diseases. For example, in vasculitis, anti-neutrophil cytoplasmic antibodies (ANCA) that are directed against proteinase 3 or myeloperoxidase are neutrophil-derived autoantigens and activated neutrophils are the main effector cells of vascular damage. In other autoimmune diseases, these neutrophil-derived neoepitopes may arise from a number of processes that include release of granule enzymes and ROS, changes in the properties of components of their plasma membrane as a result of activation or apoptosis, and via the release of Neutrophil Extracellular Traps (NETs). NETs are extracellular structures that contain chromatin that is decorated with granule enzymes (including citrullinated proteins) that can act as neo-epitopes to generate auto-immunity. This review therefore describes the processes that can result in neutrophil-mediated auto-immunity, and the role of neutrophils in the molecular pathologies of auto-immune diseases such as vasculitis, rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE). We discuss the potential role of NETs in these processes and some of the debate in the literature regarding the role of this phenomenon in microbial killing, cell death and auto-immunity.

Abbreviations: AAV, ANCA-associated vasculitis; ACPA, anti-citrullinated protein antibodies; ANCA, anti-neutrophil cytoplasmic antibodies; APRIL, a proliferation-inducing ligand; BAFF/BLyS, B-cell activating factor/B lymphocyte stimulator; EGPA, eosinophilic granulomatosis with polyangiitis; G-CSF, granulocyte-colony stimulating factor; GM-CSF, granulocyte/macrophage-colony stimulating factor; GPA, granulomatosis with polyangiitis; ICAM, intercellular adhesion molecule; IFN, interferon; IL, interleukin; JSLE, juvenile systemic lupus erythematosus; LDG, low density granulocyte; LPS, lipopolysaccharide; MMP, matrix metalloproteinase; MPA, microscopic polyangiitis; MPO, myeloperoxidase; NADPH oxidase, nicotinamide adenine dinucleotide phosphate-oxidase; NET, neutrophil extracellular trap; PAD, protein-arginine deiminase; pDC, plasmacytoid dendritic cell; PMA, phorbol 12-myristate 13-acetate; PR3, proteinase 3; RA, rheumatoid arthritis; RAGE, receptor for advanced glycosylation endproducts; RANKL, receptor activator of nuclear factor kappa-B ligand; RNP, ribonucleoprotein; ROS, reactive oxygen species; SLE, systemic lupus erythematosus; STAT, signal transducer and activator of transcription; TLR, toll-like receptor; TNF, tumour necrosis factor.

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1. Introduction

1.1. Neutrophils and host defence

Neutrophils play key roles in the control of bacterial and fungal infections, via their ability to migrate from the circulation to sites of infection, and when at these sites, to recognize and destroy the invading pathogens [1]. Neutrophils are therefore highly-specialised killing cells, containing a wide variety of degradative enzymes (e.g., proteases, hydrolases, nucleases) in their granules, plus the ability to generate reactive species (ROS) via an activated NADPH oxidase in combination with myeloperoxidase [2–4]. These cytotoxic components can, by acting together, rapidly and effectively kill a wide range of microbial targets [4]. These properties of neutrophils make them uniquely adapted for this killing role, and indeed they have the highest cytotoxic potential of all immune cells.

In order to perform this role in host defence, inactive neutrophils in the circulation must respond to regulatory or chemotactic signals (e.g., cytokines, chemokines and host- or pathogen-derived factors) and move from the circulation to the site of infection. This process involves "priming" of their functions which occurs via activation of kinase cascades, changes in the surface properties of the cells via movement of cytoplasmic granules to the cell surface and activation of de novo gene expression [5–7]. All of these mechanisms contribute to the processes that result in a "primed" neutrophil with a greater cytotoxic capacity, an extended lifespan and enhanced functions that allow these cells to mount an effective challenge during the acute inflammatory response [6]. During these processes, neutrophils themselves can secrete cytokines, chemokines and other regulatory molecules that can promote inflammation (including recruitment and activation of other neutrophils) and also regulate other elements of the immune system [8,9]. Once neutrophil function is complete (e.g., the infection is cleared) they undergo cell death by apoptosis and inflammation normally resolves [6].

1.2. Neutrophils, inflammation and inflammatory damage

Whilst this key role of neutrophils in host defence has been appreciated for many years, the ability of these cells to contribute to the tissue damage associated with inflammation and inflammatory diseases is also recognised. Observations from human studies and animal models implicate neutrophils and their products of activation (e.g., proteases and ROS) in the tissue- and organ-damage associated with human diseases, that include rheumatoid arthritis, vasculitis, chronic obstructive pulmonary disease and inflammatory bowel disease [10–13]. In such diseases, neutrophils can infiltrate tissues and become inappropriately activated, e.g. as a result of infection or via immune complexes [14] to secrete molecules that are normally retained in phagocytic vesicles following phagocytosis of pathogens [15]. These secreted molecules can attack host tissues if they overwhelm endogenous tissue levels of anti-proteinases or anti-oxidants [16].

In addition to their direct role in initiating tissue damage in inflammatory diseases, neutrophil-derived cytokines, chemokines and other regulatory molecules (e.g., eicosanoids) can also orchestrate the functions of other immune cells in these inflammatory conditions [8–10]. Far from being passive cells that can only respond to inflammatory signals by generating a cytotoxic response, it is now recognised that neutrophils are key players in the regulation of almost every element of the immune response: from control of haematopoiesis to modulation of T and B cell function [8,17]. In inflammatory diseases such as rheumatoid arthritis, a variety of cytokines and chemokines are implicated in disease pathology [18,19], and this phenomenon has formed the basis for the development of a range of anti-cytokine biologic drugs (typified by TNF inhibitors, TNFi) which can result in dramatic improvements in disease activity [20]. Neutrophil-derived cytokines may, at least in part, contribute to the dysregulated cytokine/chemokine signalling networks that characterize these diseases [8,19].

More recently, it has become recognised that neutrophils may also be the source of auto-antigens, via a number of mechanisms (Fig. 1). These include: neutrophil degranulation (which releases granule enzymes into the extracellular environment and also changes the properties of the plasma membrane); apoptosis, the process of regulated cell death, which also results in changes to the properties of the plasma membrane; neutrophil extracellular trap (NET) formation. This review will focus therefore on the processes by which neutrophils can expose or generate auto-antigens that result in the generation of autoantibodies that characterize a number of human diseases, and hence how neutrophils may contribute to immune dysregulation favouring auto-immunity. For a comprehensive review of the use of animal models to determine the role of neutrophils in autoimmune diseases, the reader is referred to [21] and the article by Lowell and co-workers in this issue of Seminars in Immunology.

1.3. Mechanisms of exposure of neutrophil-derived auto-antigens

1.3.1. Neutrophil activation and degranulation

During phagocytosis, internalised microbes or immune complexes are localized within phagolysosomes, the membranes of which contain an activated NADPH oxidase (that generates ROS) while the matrix of these phagocytic vesicles becomes enriched with activated granule enzymes (such as myeloperoxidase, defensins and proteases) following fusion of granules with the phagocytic vesicle [4]. Generally during this process of phagocytosis, very few, if any of these cytotoxic molecules are released extracellularly from the phagocytosing neutrophil. However, there are a number of circumstances in which neutrophil contents, especially granule enzymes and ROS, can be released extracellularly, and this processes can result in oxidative-modification of serum proteins to enhance their antigenicity, thereby converting them to auto-antigens (Fig. 1) [22]. Circumstances under which this secretion can occur include when the phagocytic target is too large to be ingested (e.g., a large fungal or protozoal target) or during "frustrated phagocytosis", for example, when cartilage or another surface becomes deposited with immune complexes and hence recognised by neutrophil immunoglobulin receptors [10,23]. During this latter process the concentrations of released neutrophil-derived products into this confined zone can be so high as to easily saturate endogenous levels of anti-proteinases and anti-oxidants [16].

Alternatively, when neutrophils have been "primed", for example by cytokines to alter their functional responsiveness to ligands, soluble agonists (e.g.,) soluble immune complexes or bacterial-derived peptides (of the fMet-Leu-Phe family) can induce a rapid (within minutes) and extensive release of ROS and granule enzymes into the external environment (Fig. 1) [5,24]. Activation in this way can also result in changes to the plasma membrane of the activated neutrophils and the cell surface expression of granule proteins, such as myeloperoxidase and proteinase 3 [25]. This process has been implicated in the pathogenesis of vasculitis (Fig. 2) [11]. Additionally, released neutrophil granule enzymes and ROS may modify the structures of host proteins and other targets to again alter their properties to expose neo-epitopes that may lead to loss of immune tolerance.

1.3.2. Apoptosis

Neutrophils have a very short half-life and exhibit high rates of constitutive apoptosis [26,27]. During culture *in vitro*, their half-

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